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Helicobacter pylori Does Not Require Lewis X or Lewis Y Expression To Colonize C3H/HeJ mice

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Helicobacter pylori **strains frequently express Lewis X (Le^x) and/or Le^y on their cell surfaces as constituents of the O antigens of their lipopolysaccharide molecules. To assess the effect of Le^x and Le^y expression on the ability of *H. pylori* to colonize the mouse stomach and to adhere to epithelial cells, isogenic mutants were created in which *fucT1* alone or *fucT1* and *fucT2*, which encode the fucosyl transferases necessary for Le^x and Le^y expression, were deleted. C3H/HeJ mice were experimentally challenged with either wild-type 26695 *H. pylori* or its isogenic mutants. All strains, whether passaged in the laboratory or recovered after mouse passage, colonized the mice well and without consistent differences. During colonization by the mutants, there was no reversion to wild type. Similarly, adherence to AGS and KatoIII cells was unaffected by the mutations. Together, these findings indicate that Le expression is not necessary for mouse gastric colonization or for *H. pylori* adherence to epithelial cells.**

Helicobacter pylori strains are persistent gastric colonizers of the human stomach whose presence increases the risk of peptic ulcer disease and gastric adenocarcinoma (5). These organisms are highly adapted for the human stomach, which presents formidable environmental challenges for any potential colonizer (5, 6). In addition to their conserved features, human gastric epithelial cells may express oligosaccharides corresponding to the Lewis A (Le^a), Le^b, Le^x, and Le^y histo-blood group antigens (27). *H. pylori* cells also are polymorphic in that they may express Le^x or Le^y (29, 36) and occasionally other related antigens as constituents of the O antigens of their lipopolysaccharide (LPS) molecules (22, 23). It has been suggested that expression of Le antigens may camouflage the bacterium or aid in bacterial adhesion (24). Additionally, *H. pylori* Lewis antigens undergo phase variation, that is, the random, reversible high-frequency switching of phenotype, a phenomenon which in some organisms, including *Neisseria* spp. and *Haemophilus influenzae*, contributes to virulence (1, 2, 4). Recently we have provided evidence that the host and bacterial Le phenotypes are related (7, 37), although this hypothesis has not been universally confirmed (16, 31).

Experimental challenge of mice with *H. pylori* leads to persistent infection which has many of the features of human colonization (19, 25). Mouse colonization assays have been used previously to assess the importance of various *H. pylori* genes in gastric colonization (10, 14). We sought to determine whether *H. pylori* strains deficient in Lewis antigen expression would be able to colonize mice. In previous studies, *H. pylori* strains with low levels of Lewis antigen expression were unable to colonize mice (3, 21, 25). However, the strains studied produced low overall levels of O antigens, and a *galE* mutant produced no O antigen. Thus, the lack of colonization could reflect lack of expression either of the entire O antigen or of its Le constituents specifically. We now report that mutants of *H. pylori* in which both 1,3-fucosyl transferases are mutated, leading to the absence of Le^x and Le^y expression, are able to colonize the mouse stomach in a manner essentially identical to that of the wild-type strain. The mutant and wild-type cells also bound equally to gastric epithelial cells.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains and plasmids used in this study are listed in Table 1. Stock cultures were maintained at −70°C in brucella broth (BBL Becton Dickinson Microbiology Systems, Cockeysville, Md.) supplemented with 15% glycerol. The isolates were routinely subcultured on Trypticase soy agar plates with 5% sheep blood (TSB [BBL]) in a humid microaerobic atmosphere with 5% CO_2 at 37°C for 48 h. The identity of *H. pylori* was confirmed by characteristic colony morphology and biochemical activities (35).

Construction of α-1,3-fucosyltransferase (FucT) gene mutants. Construction of the isogenic FucT mutants of wild-type *H. pylori* strain 26695, in which we interrupted either one or both copies of the FucT genes, was performed as follows. Open reading frames (ORFs) HP0379 (*fucT1*) and HP0651 (*fucT2*) were first amplified by PCR using the primers indicated in Table 2. *Eco*RI and *Xho*I restriction sites were incorporated into the 5′ ends of the primers to ensure directional cloning of the genes. The digested and purified PCR products then were cloned into pBluescript and transformed into *Escherichia coli* DH5α, and recombinants (pTT379 and pTT651) were selected on Luria-Bertani plates containing 50 μg of ampicillin per ml. Each ORF was disrupted by insertion of an antibiotic resistance cassette. An *aphA* cassette (kanamycin resistance) was released from pILL600 by *Bam*HI digestion and ligated into a *Bgl*II site at position 204 in both *fucT* ORFs. These constructs were used to transform *E. coli* DH5α

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype or characteristics	Reference
Strains		
H. pylori		
26695	Wild type	33
98-950	26695/0651::*aphA*	This study
98-1014	26695/0379::*cat*/0651::*aphA*	This study
E. coli DH5α	*endA1 hsdR17* (r_K^- m_K^+) *supE44 thi-I recA gyrA* (Nal^r) *relAl* Δ(*argG-lacZYA*) U169 *deoR* [Φ80 Δ*lac*Δ(*lacZ*)M15]	
Plasmids		
pBlueScript	ColE1 Amp^r; PCR cloning vector	
pILL600	Amp^r Km^r; source of kanamycin cassette	18
pSAT101::cm	pBlueScript, *H. pylori recA* (5′ end), Cm^r; source of chloramphenicol cassette	32
pTT379	pBlueScript, 0379	This study
pTT651	pBlueScript, 0651	This study
pTT0379K	pBlueScript, 0379::*aphA*	This study
pTT0651K	pBlueScript, 0651::*aphA*	This study
pTT0379C	pBlueScript, 0379::*cat*	This study
pTT0651C	pBlueScript, 0651::*cat*	This study

with selection on Luria-Bertani plates containing 50 μg of ampicillin per ml and 30 μg of kanamycin per ml. The plasmids containing the mutated ORF HP0379 and HP0651 were designated pTT379K and pTT651K, respectively, and used to transform *H. pylori* strain 26695 by natural transformation. After we mutated each of the two ORFs alone, we then inactivated the second FucT ORF in several single mutants. To construct plasmids in which HP0379 or HP0651 was disrupted by a *cat* (chloramphenicol resistance) cassette, the *cat* cassette was amplified from pSAT101::cm (32) by PCR, purified, digested with *Bgl*II, and ligated with *Bgl*II-digested and purified ORFs of pTT379 and pTT651. *H. pylori* strains that had a single *aphA* insertion in either HP0379 (*fucT1*) or HP0651 (*fucT2*) were transformed using plasmids pTT379C and pTT651C containing the reciprocal *cat* insertion, and recombinants were selected on TSB plates containing both kanamycin and chloramphenicol. Correct insertion of the resistance cassettes into the intended target gene was confirmed by PCR. Silver staining and Western blotting of proteinase K-treated whole-cell lysates (22, 26) confirmed that the LPS of each of the mutants contained intact O antigens and that the Lewis antigen, when present, migrated on polyacrylamide gel electrophoresis as part of the O antigen.

Animals and housing. Female C3H/HeJ mice approximately 3 to 5 weeks old were purchased from Jackson Laboratory (Bar Harbor, Maine). Up to six animals per solid-bottom cage were housed in the Animal Care Facilities of Vanderbilt University in a room with a 12-h light-dark cycle at 21 to 22°C, and animals were fed a standard commercial rodent chow. Access to food and water was free throughout all experiments. No special pretreatment (such as acid inhibition or antibiotics) was used before orogastric *H. pylori* inoculation or before the animals were sacrificed. All experiments and procedures carried out with the animals had been approved by the Institutional Animal Care Committee of Vanderbilt University.

***H. pylori* inoculation and animal sacrifice.** C3H/HeJ mice were inoculated with cells of wild-type strain 26695 or its isogenic single (Δ*fucT2*), and double (Δ*fucT1/2*) mutants. The bacteria had been taken from the freezer immediately prior to mouse challenge. The mice received inocula of 10^9 to 10^{10} bacterial cells three times in a 3- to 4-day period. Mice were sacrificed at predetermined times up to 12 weeks after challenge. Animals were anesthetized in a CO_2 chamber and sacrificed by cervical dislocation. At the time of sacrifice, blood was collected, and serum was obtained by centrifugation and frozen at −20°C for later determination of serum antibodies. The stomach was harvested, and one-third was macerated into a suspension for quantitative *H. pylori* culture, as described previously (38). The macerated specimens were inoculated onto Trypticase soy agar–5% sheep blood plates containing vancomycin, nalidixic acid, bacitracin, and amphotericin B in the concentrations described previously (38). In each case, colonies were identified as *H. pylori* based on their resistance to the antibiotics, characteristic morphology on plates, and the urease and oxidase activities of multiple colonies. Isolates from these studies (mouse passaged) were characterized and then, after three or fewer in vitro passages, were used to challenge fresh mice.

Characterization of *H. pylori* isolates. For determination of Le^x and Le^y expression, *H. pylori* isolates grown for 48 h on TSB plates were harvested and washed twice with 3 ml of 0.15 M NaCl per plate. Le^x and Le^y antigen expression was determined by specific enzyme-linked immunosorbent assays (ELISAs) as described previously (35). To determine genotypes of *H. pylori* isolates recovered from mice, PCR was done using primers specific for *fucT1* and *fucT2* as shown in Table 2.

Serum antibodies to *H. pylori*. For evaluation of the time course of the systemic immune response to *H. pylori*, sera from the sacrificed mice were studied. Levels of immunoglobulin G (IgG) antibody directed toward *H. pylori* whole-cell antigens in the serum specimens were measured by ELISA, as described previously (35). Seroconversion to *H. pylori* was defined as a serum IgG response to the *H. pylori* antigens that was greater than the mean plus three standard deviations for the unchallenged mice.

Binding of *H. pylori* to gastric epithelial cells. KatoIII and AGS-CDM cells are gastric adenocarcinoma cell lines obtained from the American Type Culture Collection. The cells were grown in RPMI 1640 medium (Gibco BRL, Grand Island, N.Y.) containing 10% fetal bovine serum in polystyrene tissue culture flasks at 37°C. AGS-NY2 cells were kindly provided by Steven Moss and grown in Ham's F12 medium. In some experiments, epithelial cells were treated with 100U of gamma interferon (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) for 48 h before use and then washed twice in phosphate-buffered saline containing bovine serum albumin (1%) and sodium azide (0.02%). To evaluate binding of *H. pylori*, adherent cells from control or gamma interferon-treated cultures were collected by gentle scrapping with a rubber policeman and then washed by centrifugation at 200 × *g* for 5 min twice before being resuspended in medium at the appropriate concentration for use in the experiments. Cell viability was assessed by trypan blue exclusion. COS-1 and ID12 cells were cultured in Dulbecco's modified Eagle's medium with Geneticin (G418 sulfate; Gibco BRL) as described previously (8). Binding of *H. pylori* to the surface of epithelial cells was evaluated by flow cytometry using a modification of techniques described elsewhere (12; Y. Minohara and P. B. Ernst, unpublished data). Briefly, the test strains of *H. pylori* were labeled with PKH26 (Sigma, St. Louis, Mo.). Gastric epithelial cells were incubated with labeled *H. pylori* cells for 1 h at room temperature and washed three times with phosphate-buffered saline. Subsequently, the cells were resuspended in 400 ml of 1% paraformaldehyde and analyzed by flow cytometry (FACScan; Becton Dickinson, San Jose, Calif.) and the binding was expressed as the relative mean fluorescence intensity (12).

TABLE 2. Oligonucleotide primers used in this study

Primer designation	*H. pylori* gene(s)	Nucleotide position[a]	Orientation[b]	Primer sequence (5′→3′)[c]
F1-f	HP0379, HP0651	1 to 24	F	GGCGGGAATTCATGTTCCAACCCCTATTAGACGCC
F1-r	HP0379	1251 to 1275	R	GGCGGCTCGAGCAAACCCAATTTTTTAACCAACTTT
F2-r	HP0651	1404 to 1428	R	GGCGGCTCGAGCTTTTTAACCCATCTCCTTATGGGTG
F1-FO	HP0379	−200 to −179	F	GCGTGCTAGGGTTTTATTCGG
F1-RO	HP0379	+1334 to +135.	R	ATTAGGGGCCAATATCGCTGG
F2-FO	HP0651	−112 to −91	F	AGAGGTTTTAAAACGCAACGC
F2-RO	HP0651	+1498 to +151	R	ACATGCTCAAAAACCCCACGC

[a] For primers F1-1, F1-r, and F2-r, location refers to position within ORFs in strain 26695. For primers F1-FO, F1-RO, F2-FO, and F2-RO, − or + indicates position upstream or downstream from the nucleotide of the initiation codon.
[b] F, forward; R, reverse.
[c] Added restriction sites are underlined: GAATTC, *Eco* RI; CTCGAG, *Xho*I.

TABLE 3. Lewis antigen expression by wild-type *H. pylori* strain 26695 and isogenic Δ*fucT* mutants after mouse challenge

Passage	Bacterial genotype	Wk	No. of mice	Mean optical density units ± SD (range)[a]	
				Le^x	Le^y
Laboratory	Wild type	Prechallenge	NA[b]	1,881 ± 179 (1,734–2,146)	649 ± 67 (577–722)
		2	3	1,996 ± 108 (1,503–2,399)	583 ± 58 (349–1,057)
		6	3	352 ± 58 (250–446)	403 ± 139 (192–907)
		12	NA	NA	NA
	Δ*fucT2*	Prechallenge	NA	206 ± 24 (180–226)	426 ± 34 (388–456)
		2	5	237 ± 73 (36–494)	348 ± 204 (126–419)
		6	4	355 ± 89 (45–509)	426 ± 108 (224–993)
		12	4	613 ± 190 (3–1,111)	838 ± 226 (6–1,474)
	Δ*fucT1/2*	Prechallenge	NA	7 ± 2 (0–17)	−2 ± 1 (0–12)
		2	4	3 ± 5 (0–16)	−3 ± 10 (−7–16)
		6	4	4 ± 4 (1–12)	3 ± 5 (0–12)
		12	4	10 ± 4 (6–18)	1 ± 2 (0–4)
Mouse[c]	Wild type	Prechallenge	NA	1,143 ± 197 (923–1,420)	146 ± 33 (106–196)
		2	4	399 ± 130 (134–636)	99 ± 58 (63–216)
		4	4	550 ± 134 (340–779)	164 ± 67 (98–371)
		12	4	796 ± 247 (385–1,173)	208 ± 83 (97–374)
	Δ*fucT2*	Prechallenge	NA	314 ± 98 (106–665)	90 ± 58 (52–193)
		2	5	475 ± 90 (257–695)	136 ± 46 (71–222)
		4	4	505 ± 109 (295–713)	157 ± 54 (84–267)
		12	4	198 ± 218 (−14–608)	108 ± 112 (−12–328)
	Δ*fucT1/2*	Prechallenge	NA	6 ± 21 (−12–42)	8 ± 19 (−3–42)
		2	4	3 ± 5 (−3–16)	−3 ± 10 (−17–16)
		4	4	0 ± 14 (−14–53)	0 ± 24 (−13–63)
		12	4	−1 ± 4 (−11–3)	14 ± 26 (−19–98)

[a] Values for one to five single colonies picked from preinoculation culture or from primary culture plate for each animal tested.
[b] NA, not applicable.
[c] Mouse-passaged strains were isolated from the animals used for laboratory passage and were used to challenge fresh mice after three or fewer in vitro passages.

RESULTS

Le^x and Le^y expression of Δ*fucT1/2* and Δ*fucT2* mutants. Using antigen-specific ELISAs for analysis of isolates before mouse challenge, the Δ*fucT2* mutant was shown to have greatly reduced Le^x expression and slightly reduced Le^y expression compared to the wild-type strain (Table 3). The Δ*fucT1/2* mutant showed values of less than 10 optical density units for both Le^x and Le^y, which confirms that inactivation of both α-1,3-fucosyltransferase genes blocks Le^x and Le^y expression (34). Since the test strains expressed O antigens but the Δ*fucT1/2* mutant was deficient in Le expression, we now could specifically examine the role of Le expression in mouse gastric colonization.

Mouse challenge with laboratory-passaged *H. pylori* 26665 wild-type and mutant strains. To examine the effect of elimination of the Lewis phenotype on gastric colonization in mice, laboratory-passaged wild-type 26695 and its isogenic Δ*fucT2* and Δ*fucT1/2* mutants were orally inoculated into C3H/HeJ mice. The results of these experimental challenges are summarized in Table 4. Compared with colonization by the wild-type 26695 strain (60%), challenge with the *fucT* mutants yielded higher colonization rates (87% for Δ*fucT2* and 80% for

TABLE 4. Gastric colonization of C3H/HeJ mice with wild-type *H. pylori* strain 26695 or isogenic Δ*fucT* mutants detected by culture

Passage	Bacterial genotype	No. of infected animals (mean $\log_{10}$ *H. pylori* CFU ± SD[a]) at wk:			
		2 (n = 5/strain)	6 (n = 5/strain)	12 (n = 5/strain)	Total (n = 15/strain)
Laboratory	Wild type	3 (1.90 ± 0.10)	3 (1.93 ± 0.21)	NE[b]	6[c] (1.92 ± 0.15)
	Δ*fucT2*	5 (3.58 ± 0.75)	4 (3.35 ± 0.52)	4 (3.40 ± 0.14)	13 (3.45 ± 0.52)
	Δ*fucT1/2*	4 (4.05 ± 1.00)	4 (3.60 ± 0.08)	4 (3.73 ± 0.10)	12 (3.79 ± 0.56)
Mouse	Wild type	4 (1.61 ± 0.50)	4 (2.32 ± 1.03)	4 (3.02 ± 0.36)	12 (2.31 ± 0.87)
	Δ*fucT2*	5 (3.18 ± 0.33)	4 (2.57 ± 0.60)	4 (1.83 ± 0.96)	13 (2.58 ± 0.87)
	Δ*fucT1/2*	4 (1.34 ± 0.26)	4 (1.91 ± 0.51)	4 (2.76 ± 0.94)	12 (2.02 ± 0.88)

[a] The calculation includes only mice in which colonization was detected by culture.
[b] NE, not examined.
[c] Only 10 mice were challenged in total, since there was no 12-week challenge.

Δ*fucT1/2*). The *fucT* mutants also showed higher levels of colonization at 2 and 6 weeks (between 10^3 and 10^5 CFU/stomach) than the wild-type strain (between 10^1 and 10^2 CFU/stomach) (Table 4). Thus, elimination of Le^x and Le^y expression did not interfere with the ability of laboratory strain 26695 to colonize the stomachs of mice.

Mouse challenge with mouse-passaged *H. pylori* 26665 wild-type and mutant strains. To determine whether the results with the laboratory-passaged strains adequately reflected the situation for strains that had been passed in vivo, next we examined colonization by strains recovered from mice in the previous experiments. We found that the mouse-passaged Δ*fucT* mutants colonized the challenged mice as frequently as did the mouse-passaged wild-type parental strain (Table 4), and there were no significant differences in the frequency of colonization up to 12 weeks, when the experiment ended (80% for wild type, 87% for Δ*fucT2*, and 80% for Δ*fucT1/2*). Comparing sequential colonization rates, there was no evidence of spontaneous *H. pylori* clearance from the animals for up to 12 weeks, regardless of the challenge strain. The mean levels of colonization determined by quantitative culture were between 10^1 and 10^4 CFU/stomach, and there was no substantial difference between the wild type and the Δ*fucT2* or Δ*fucT1/2* mutant (Table 4). Since one possibility to explain the robust colonization by the mutant strains is reversion to wild type, we performed PCR analysis of the HP0379 and HP0651 genes of the Δ*fucT2* and Δ*fucT1/2* isolates after mouse passage. Both the laboratory- and mouse-passaged strains showed patterns identical to those of the preinoculation mutant strains, indicating the retention of the antibiotic resistance cassette (data not shown). These results indicated that there had been no reversion to wild type.

Le^x and Le^y expression by wild-type *H. pylori* and isogenic mutants after mouse passage. To determine whether the expression of Le^x and Le^y of the individual *H. pylori* strains had changed during mouse passage, perhaps reflecting selection for particular phenotypes, Le typing was performed on five single colonies from each of the isolates obtained prechallenge and from each sacrifice time point (Table 3). In both experiments, involving the laboratory- or mouse-passaged wild-type strains, expression of Le^x declined compared with that prechallenge, but the Le^y expression changed little. The Δ*fucT1/2* mutants showed no measurable Le^x or Le^y expression through the 12 weeks of colonization. The lack of Le expression in these mutants, consistent with the genotypic evidence of the stability of the insertion mutations, indicated no reversion to wild type.

Serum antibody response to *H. pylori*. The serum antibody response to *H. pylori* whole-cell antigens was monitored from prechallenge to 12 weeks postchallenge in the 85 animals. The colonized animals showed progressive increases in the serum IgG response to *H. pylori* antigens through the 12-week experiments, as shown in the studies of the mice challenged with the mouse-passaged strains (Fig. 1). There were no significant differences in response based on the Lewis status of the challenge strain. For 20 mice that were *H. pylori* culture positive at 12 weeks postchallenge, there was no correlation between the antibody responses and bacterial CFU (data not shown). All 20 had seroconverted to the *H. pylori* antigens, which is consistent with their persistent infection. Since seroconversion was sensitive for detecting colonization, when we applied the same seroconversion criterion to the 17 culture-negative mice, we found that all 17 mice (100%) also met the criterion. In total, based on either the culture (80%) or seroconversion (96.5%) criterion, all (100%) of the 85 C3H/HeJ mice challenged with *H. pylori* in this study had become infected, regardless of the *H. pylori* genotype and Le expression (Tables 3 and 4).

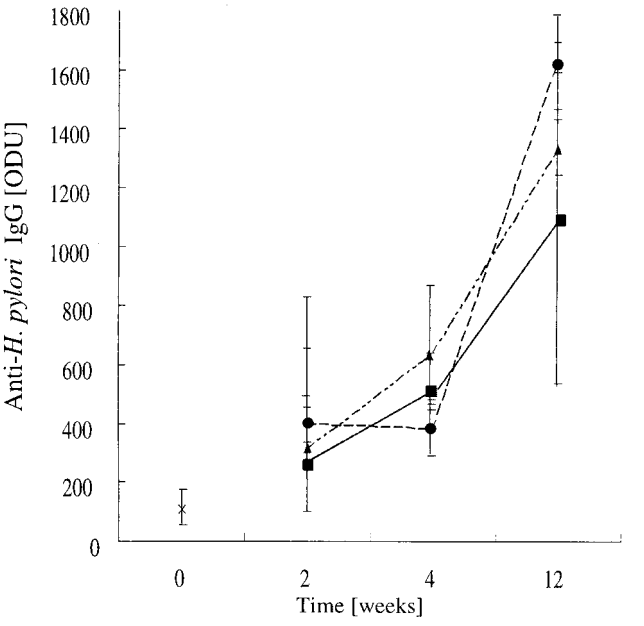

FIG. 1. Serum IgG responses to *H. pylori* whole-cell antigens in C3H/HeJ mice following challenge with wild-type (■) and isogenic Δ*fucT2* (●) and Δ*fucT1/2* (▲) mutant mouse-passaged *H. pylori* strains. ×, negative control. Error bars indicate standard deviations. ODU, optical density units.

Effect of Le expression on *H. pylori* binding to gastric epithelial cells. Since *H. pylori* naturally colonizes humans and not mice, we next performed in vitro experiments to assess the effect of Le expression on binding of *H. pylori* cells to human gastric epithelial cells. The assays entail labeling viable *H. pylori* cells with a chromophore, incubating with the epithelial cells at various bacteria/cell ratios, and, after washing, assaying the epithelial cells for fluorescence by flow cytometry. Studies using AGS-CDM, AGS-NY2, and KatoIII cells showed similar results; for bacteria/cell ratios of between 1:1 and 100:1, there was essentially no difference between the 26695 wild-type and Le knockout strains (Fig. 2). Treatment of KatoIII cells with gamma interferon increased the binding of both the wild-type and Δ*fucT1/2* knockout strains, but in the same proportions (data not shown). Transfection of Cos cells ID12, which express class II major histocompatibility complex, slightly increased binding at the 1:1 ratio for both the wild type and the Δ*fucT1/2* mutant but had no effect on binding at the 10:1 or 100:1 bacteria/cell ratio (data not shown). In total, Le expression had no significant effect on *H. pylori* binding in any of the cell binding assays studied.

DISCUSSION

To establish the role of Le expression in the ability of *H. pylori* strains to colonize the murine stomach, we created mu-

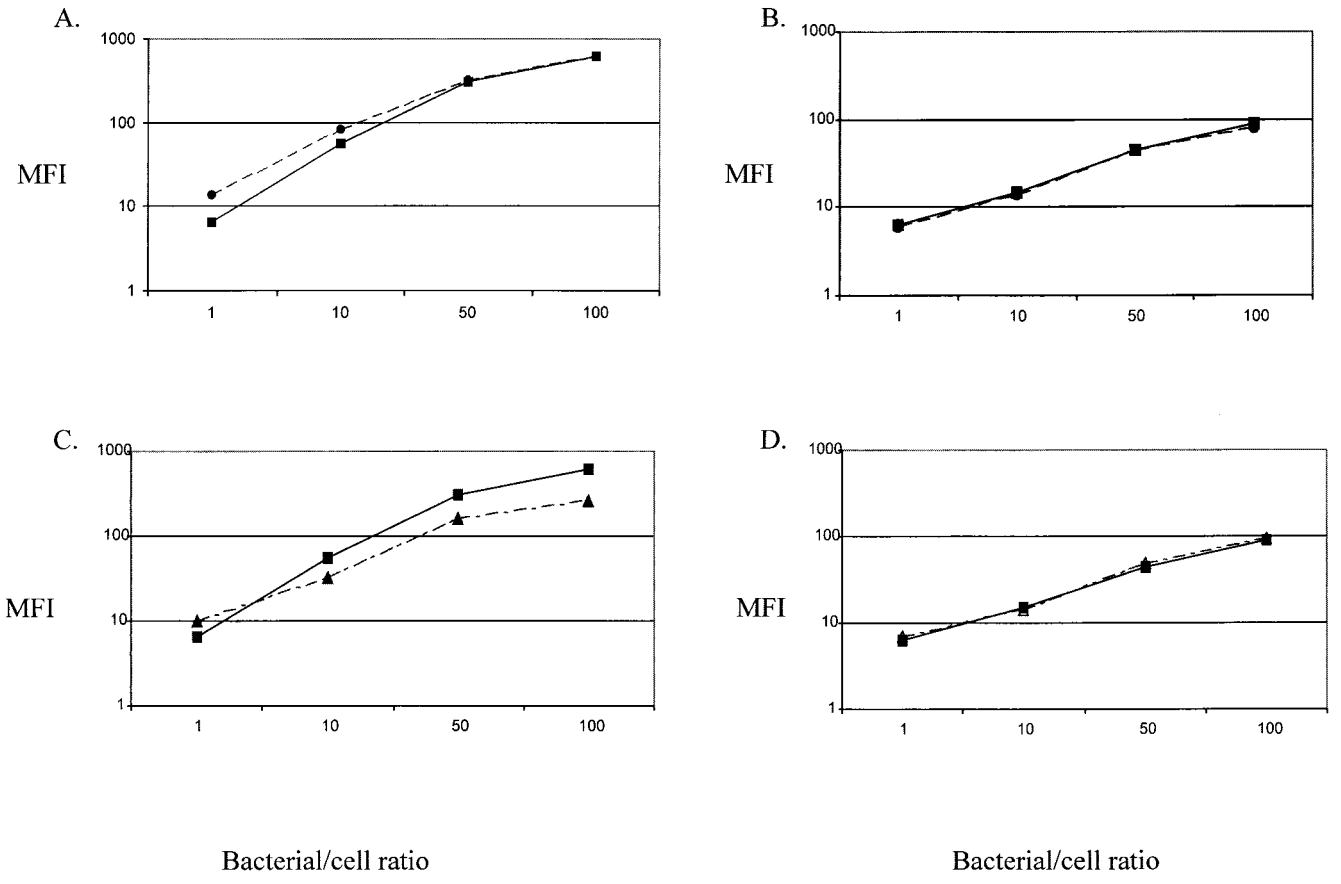

FIG. 2. Binding of *H. pylori* strains to gastric epithelial cells (AGS-NY2 [A and C] and KatoIII [B and D]). Each panel compares binding of wild-type strain 26695 (solid lines) with that of its isogenic Δ*fucT2* (A and B) or Δ*fucT1/2* (C and D) mutant (dashed lines). Bacteria/cell ratios ranged from 1:1 to 1:100, and binding was estimated as the mean fluorescence intensity (MFI) by flow cytometry. Results are representative of those from two separate experiments.

tants with mutations in one or both of the 1,3-fucosyltransferase genes that are required for expression of both Le^x and Le^y (13, 20, 34). As expected, when both *fucT1* and *fucT2* both were inactivated, Le expression was blocked. In the present studies, we showed that this mutant genotype and its resulting phenotype persisted when the strains were passed in vivo for 12 weeks.

By using a variety of model systems, *H. pylori* virulence factors that affect the organism's ability to colonize the host have been identified (10). Such factors include motility, urease function, and superoxide dismutase activity (8, 9, 28). The results of this study clearly show that Le expression is not required for *H. pylori* colonization of C3H/HeJ mice, at least for up to 12 weeks. Since the initial studies were performed with laboratory-passaged strains that had had an unknown number of in vitro passages, we also recovered *H. pylori* cells from mice that had undergone challenge and used these mouse-passaged strains to assess whether in vivo passage led to any substantial differences. One limitation is that mouse passage may have selected for *H. pylori* cells better able to colonize the mouse stomach; however, the *fucT* genotypes and Le phenotypes were essentially unchanged, indicating that there had not been either reversion to wild type or upregulation of expression by a complementary mechanism. Recently, Suresh and colleagues (30) reported that a wild-type *H. pylori* strain lacking Le^x and Le^y expression was able to colonize both C57/BL6 and BALB/c mice. Although the genotype of that strain was not reported and the phenotype of the cells recovered from the mice was not determined, the present results both confirm and extend that work. Taken together, these results clearly indicate that *H. pylori* strains not expressing either Le^x or Le^y can colonize mice, at least for a period of 12 weeks. In studies reported by Martin et al. (21), a double *fucT* mutant lacking Le^x or Le^y expression colonized outbred HSD/ICR mice substantially more poorly than did its parental strain. Whether differences in the background *H. pylori* strains used or in the host mouse genotype (outbred versus C3H/HeJ in our studies) contributed to the differences observed remains to be determined. The C3H/HeJ LPS-nonresponder mice, with a mutation in Toll-like receptor 4, which recognizes bacterial LPS (17), may be more permissive for *H. pylori* colonization than most other mouse strains.

The ability of strains lacking Le^x and Le^y expression to colonize mice suggests several hypotheses. First, since some *H. pylori* strains recovered from humans do not express Le^x or Le^y and apparently are fully capable of colonizing humans (31), Le expression is not required for colonization of the mammalian stomach and has other functions for the bacterial cell. Alter-

natively, 12 weeks is not a sufficient indicator of long-term colonization, and eventually strains lacking Le^x or Le^y expression will be cleared from the host. However, up to 10% of *H. pylori* strains isolated from humans have no detectable Le expression; this is especially notable in strains lacking the *cag* island (35). Our previous studies (39) and that of Rasko et al. (26) show that humans can be colonized by genetically indistinguishable strains that vary in Lewis antigen expression; these clonal variants also illustrate that Le expression is not required for colonization of humans. However, since murine epithelial cells do not normally express Le^b (15), this work is not fully analogous to the situation in primates, in which *H. pylori* expression of Le^x and/or Le^y generally mirrors that of the host (7, 37). An alternative hypothesis is that Le expression facilitates *H. pylori* binding to host epithelial cells (11). The results of our in vitro studies (Fig. 2) do not support that hypothesis. However, although several different cell lines were examined, with and without cytokine stimulation, these were transformed cells and may have lacked expression of critical ligands.

Together, these studies and observations in humans of *H. pylori* cells lacking Le expression indicate that *H. pylori* expression of Le^x or Le^y is not required for gastric colonization, at least over relatively short periods of time (weeks to months). These observations do not refute the hypothesis that Le expression is a bacterial phenotype subject to ongoing selection and that particular levels are adaptive among competing *H. pylori* strains for occupation of microniches. If the incremental fitness associated with expression of the phenotype most appropriate for a particular host or microniche was small (35), then the effects of this difference might not be evident except over long periods of observation.

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